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Functional Characterization and Expression of a Cytosolic Iron-Superoxide Dismutase from Cowpea Root Nodules

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PLANTS INTERACTING WITH OTHER ORGANISMS

Functional Characterization and Expression of a Cytosolic Iron-Superoxide Dismutase from Cowpea Root Nodules[1],[2]

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Abstract: An iron-superoxide dismutase (FeSOD) with an unusual subcellular localization, VuFeSOD, has been purified from cowpea (*Vigna unguiculata*) nodules and leaves. The enzyme has two identical subunits of 27 kD that are not covalently bound. Comparison of its N-terminal sequence (NVAGINLL) with the cDNA-derived amino acid sequence showed that VuFeSOD is synthesized as a precursor with seven additional amino acids. The mature protein was overexpressed in *Escherichia coli*, and the recombinant enzyme was used to generate a polyclonal monospecific antibody. Phylogenetic and immunological data demonstrate that there are at least two types of FeSODs in plants. An enzyme homologous to VuFeSOD is present in soybean (*Glycine max*) and common bean (*Phaseolus vulgaris*) nodules but not in alfalfa (*Medicago sativa*) and pea (*Pisum sativum*) nodules. The latter two species also contain FeSODs in the leaves and nodules, but the enzymes are presumably localized to the chloroplasts and plastids. In contrast, immunoblots of the soluble nodule fraction and immunoelectron microscopy of cryo-processed nodule sections demonstrate that VuFeSOD is localized to the cytosol. Immunoblot analysis showed that the content of VuFeSOD protein increases in senescent nodules with active leghemoglobin degradation, suggesting a direct or indirect (free radical-mediated) role of the released Fe in enzyme induction. Therefore, contrary to the widely held view, FeSODs in plants are not restricted to the chloroplasts and may become an important defensive mechanism against the oxidative stress associated with senescence.

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